

Development of a better and cheaper approach for fabrication of epidermal sheets used for wound healing

About 1% of the world population are affected by pressure and diabetic foot ulcers and around 11 million people are injured by thermal burns each year.

Cell therapy is a promising therapeutic approach for the treatment of several skin defects, including burns, scars, trauma, and diabetic leg ulcers. In vitro keratinocyte culture including autologous/allograft cultured keratinocytes has been applied since 1981 for treatment of ulcers, burn patients and acute and chronic wounds. However, inappropriate conditions of cultivation, can lead to decreased proliferation and early differentiation, limiting the application of cell therapy.

Three main approaches have been used for fabrication of epidermal sheets including 1) use of human feeder layer which is difficult to culture, 2) feeders composed of 3T3 cells which are considered as xenograft, and 3) applying a serum-free and xenograft-free media and collagen/fibrin matrix which are not easily accessible and cheap.

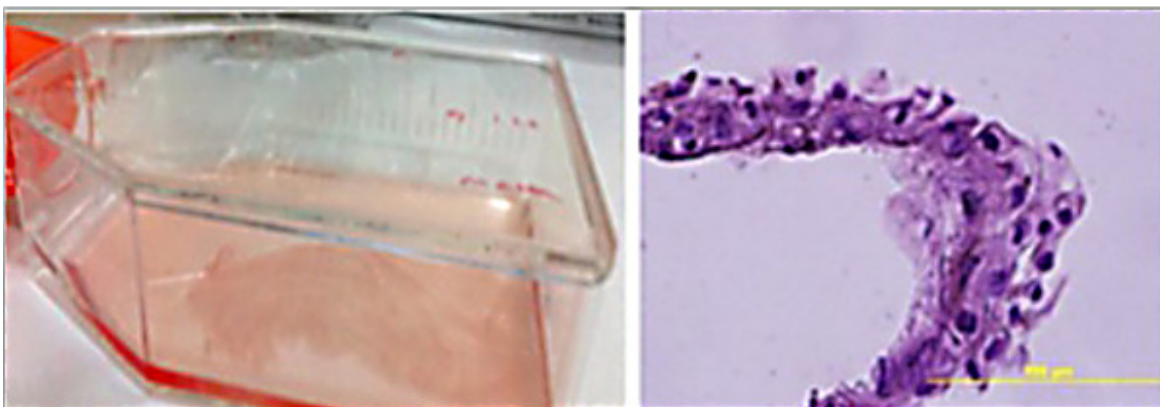


Fig. 1. Fabrication of human epidermal keratinocyte sheets in the test medium. Macroscopic view (left) and H&E-stained sections (right) of harvested cell sheets are shown.

Mesenchymal Stem Cells (MSCs) isolated from adult adipose tissue and bone marrow are self-renewing cells that can differentiate into adipocytes, osteoblasts, and chondrocytes. MSCs can reduce the inflammation, induce cell proliferation and migration, as well as angiogenesis and release paracrine signaling molecules involved in accelerating wound healing.

The studies have shown that paracrine molecules secreted from MSCs can induce the proliferation of keratinocytes, dermal fibroblasts and endothelial cells. MSC-conditioned medium is composed of various growth factors, cytokines and chemokines such as VEGF, PDGF, bFGF, EGF, KGF and TGF- β .

Considering these tremendous paracrine effects of MSCs, in this study we aimed to verify the possibility of using MSC-conditioned medium for keratinocytes culture and fabrication of an epidermal sheet suitable for

grafting and therapeutic use, with focusing on the preservation of stem cells and their differentiation potential in fabricated sheets.

Through this investigation, we introduced a new approach for culturing isolated keratinocytes in vitro to generate epidermal keratinocyte sheets without using a feeder layer. In this study, Ad-MSC conditioned medium was used instead of commercial media for keratinocyte culture. The expression of several stem/progenitor cells (*P63*, *K19* and *K14*) and differentiation (*K10*, *IVL* and *FLG*) markers was examined using quantitative RT-PCR on days 7, 14, and 21 of culture. P63 and $\alpha 6$ integrin expression were also evaluated via flow cytometry. The results were compared with the control group including keratinocytes cultured in EpiLife medium and our data indicated that this Ad-MSC conditioned medium is a good alternative for keratinocyte cultivation and producing epidermal sheets for therapeutic and clinical purposes.

All in all, our study shows evidence that Ad- MSC conditioned medium results in high expression of stem/progenitor markers and similar expression of differentiation markers in cultured keratinocytes as compared to EpiLife-control, which restores the proliferation and migration of these cells.

The fabricated epidermal sheets from these keratinocytes are promising candidates for pre-clinical applications.

Other advantages of this approach include reduced cost of cell sheet fabrication and overcoming the requirement for feeder cells which makes it xeno-free.

According to the Ad-MSC immunomodulatory properties, the keratinocytes cultured in this medium may be safe enough to transplant.

Further in vivo and in vitro studies to fully confirm the effects of MSC-conditioned medium on wound repair and validate the clinical safety of the fabricated cell sheets are required before clinical applications.

Halimeh Hassanzadeh^{1,2}, Maryam M. Matin^{1,2,3}, Hojjat Naderi-Meshkin¹, Hamid Reza Bidkhor¹, Mahdi Mirahmadi¹, Mahmood Raeesolmohaddeseen¹, Nasser Sanjar-Moussavi⁴, Ahmad Reza Bahrami^{1,2,3}

¹*Stem Cell and Regenerative Medicine Research Group, Iranian Academic Center for Education, Culture and Research (ACECR), Khorasan Razavi Branch, Mashhad, Iran*

²*Department of Biology, Faculty of Science, Ferdowsi University of Mashhad, Mashhad, Iran*

³*Novel Diagnostics and Therapeutics Research Group, Institute of Biotechnology, Ferdowsi University of Mashhad, Mashhad, Iran*

⁴*Department of General Surgery, Islamic Azad University Mashhad Branch, Mashhad, Iran*

Publication

[Using paracrine effects of Ad-MSCs on keratinocyte cultivation and fabrication of epidermal sheets for improving clinical applications.](#)

Hassanzadeh H, Matin MM, Naderi-Meshkin H, Bidkhor HR, Mirahmadi M, Raeesolmohaddeseen M, Sanjar-Moussavi N, Bahrami AR
Cell Tissue Bank. 2018 Dec